terms." The Office Action asserts that examples of such terms are cited in the rejection under 35 U.S.C. § 112, second paragraph. The Office Action further asserts that the specification should be revised. Applicants respectfully traverse.

Applicants respectfully point out that 35 U.S.C. § 112, first paragraph, states, "The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same ...". As stated below in Applicants' comments concerning the rejection under 35 U.S.C. § 112, second paragraph, terms such as "derivatives", "mutants", or "fragments" as they apply to compounds such as avidin or streptavidin are understood by those of skill in the art. Those of skill in the art would readily understand what kinds of derivatives, mutants, or fragments would be useful and conventional in the field pertaining to the claimed invention.

The M.P.E.P. states that some latitude in the manner of expression and aptness of terms should be permitted even though the claim language is not as precise as the Examiner might desire. *See M.P.E.P. § 2173.02*. In addition, the breadth of a claim is not to be equated with indefiniteness. *See M.P.E.P. § 2173.04*. Applicants assert that those of skill in the art would readily understand the teachings of Applicants' specification. Accordingly, Applicants respectfully request that the objection be reconsidered and withdrawn.

* * *

Claim 27 is objected to because a multiple dependent claim cannot depend from any other multiple dependent claim.

In response, claim 27 has been amended to no longer depend from multiple dependent claim 26. Accordingly, the objection is overcome and its withdrawal is respectfully requested.

* * *

Claims 4-10, 14, 15, 17-19, 21, 23, and 30 are rejected under 35 U.S.C. § 112, second paragraph as being indefinite. Applicants respectfully traverse.

In preparing this Amendment, Applicants have carefully reviewed the claims, taking into consideration the various issues raised by the Examiner in the Office Action. In general, the newly added claims have been prepared in a manner to address the Examiner's concerns regarding clarity as set forth in the Office Action. In particular, claims previously reciting relative terms, improper Markush language and broad and narrow ranges (including claims reciting the term "preferably") have been corrected. Moreover, new claims have been presented which recite the narrow ranges from the original claims. In addition, claim 19 has been amended to clarify that it refers to a reagent and not a method.

With regard to the terminology "derivatives, mutants, and fragments", "essentially the same binding function", and "means", Applicants respectfully assert that

those of skill in the art would readily understand what is intended by these phrases and terms. As stated above, some latitude in the manner of expression and aptness of terms should be permitted. In addition, the breadth of a claim is not to be equated with indefiniteness. Those of skill in the art would readily understand what kinds of derivatives, mutants, or fragments would be useful and conventional in the field pertaining to the claimed invention. In addition, those of skill in the art would understand that "essentially the same binding function" means compounds that have essentially the same binding function as biotin. This phrase is intended to encompass binding between compounds closely related to biotin and avidin which bind strongly enough to be useful in the claimed invention. Finally, a "means" for return of whole blood or plasma to the body during extracoporeal treatment would be easily understood by the skilled artisan. Examples of such a means include a pump.

It is respectfully submitted that the claims, including the newly added claims, fully comply with the requirements of 35 U.S.C. § 112, second paragraph. Reconsideration and withdrawal of the rejection is respectfully requested.

* * *

Claims 1-25 are rejected under 35 U.S.C. § 102(b) as being anticipated by Wilbur et al. (WO 97/29114). Applicants respectfully traverse.

The Office Action asserts that Wilbur teaches every element of the claimed invention. Thus, the Office Action concludes that Wilbur anticipates the claimed invention.

Wilbur, on page 13, also describes a trifunctional moiety which is linked to the biotin molecule via diaminopropane. However, contrary to the reagent of Applicants' invention, the trifunctional structure described in Wilbur is included to improve the solubility and not to bind to a biomolecule. None of the structures described by Wilbur contain the three essential moieties recited in Applicants' invention, namely, an affinity label, an effector, and a biomolecule reactive moiety. The diaminopropane of Wilbur can not be regarded as a biomolecule and is not in itself reactive with functional groups normally present on biological molecules. Furthermore, none of the structures described by Wilbur contain any protection against biotinidase and are therefore not particularly useful *in vivo* or in the presents of plasma *in vitro*.

Applicants' invention provides a reagent having three different moieties (an affinity label, an effector, and a biomolecule reactive moiety) each serving a specific need of simultaneously labeling of targeting molecules for in vivo diagnostic and therapeutic applications in conjunctions with extracoporeal removal of non-targeted molecules from blood circulation. As detailed above, Wilbur neither teaches nor describes such a reagent.

The amendments to the claims and above remarks overcome the rejection. Thus, reconsideration and withdrawal are respectfully requested.

* * *

Claims 1-26 and 28-30 are rejected under 35 U.S.C. § 103(a) as being obvious based on Wilbur et al. in view of Yau et al. (U.S. Pat. No. 5,541,287) or Theodore et al. (U.S. Pat. No. 5,578,287) and Maddock (U.S. Pat. No. 5,474,772). Applicants again respectfully traverse.

The Office Action asserts that Wilbur teaches every element of the claimed invention except a method of diagnosing or treatment and kits. The Office Action also asserts that Yau, Theodore, and Maddock teach diagnosing and treatment methods and kits. The Office Action thus concludes that it would have been obvious to combine the teachings of the prior thereby rendering Applicants' invention obvious.

The deficiencies of Wilbur have been discussed above. Applicants' invention solves a fundamental problem in the immunotargeting field. In these types of applications the biomolecule (i.e. an antibody) serves the purpose of making an effector (i.e. a radionucleotide, cell toxin, or drug) specific for a particular cell (i.e. tumor cells). Administration of the effector without the biomolecule, requires a high concentration of the toxic biomolecule in the blood circulation to reach sufficiently high concentration in the tumorous tissue. However, administering the effector without the biomolecule will, at therapeutic doses, cause unacceptable side effects, particularly in the bone marrow.

Even if the effector is linked to a biomolecule and biotin unacceptable side effects can occur unless the effector causing the side effect can be removed from circulation. Most potentially successful targeting biomolecules are internalized into the tumor cell through endocytosis where the protein structure is enzymatically digested by proteolytic enzymes leaving the effector separated from the biomolecule and biotin and allowing the effectors to leak out of the cells. Removal from blood circulation cannot be achieved unless the effector is directly linked to an affinity ligand (i.e. biotin) through a stable linkage. Thus, the present invention solves the problem of efficiently removing toxic therapeutics from the blood circulation regardless of the fate if the biomolecule (targeting molecule).

In addition, consecutive labeling of effector and affinity groups to the targeting molecule will render a mixture of biomolecules exhibiting the two moieties in various ratios. Statistically, a portion of the conjugated biomolecules will contain a high number of effectors but hardly any affinity ligands and these effectors can therefore not be removed because they lack a sufficient number of affinity ligands. Thus, to limit the number of non-removable effectors a higher degree of affinity ligands than would otherwise be required has to be attached. The present invention also solves the problem of heterogeneity of the conjugated molecule.

Finally, substitution of biomolecules normally leads to a partial loss of biological activity. This loss of biological activity is often directly related to the umber of substituents. In this case, more than twice the number of effector moieties and affinity

ligands are separately linked to the functional groups on the biomolecules than need to be substituted leading to a higher degree of structural as well as functional alteration to the biomolecule. in contrast, the biomolecules of the present invention are more likely prone to retain the biological activity of the biomolecule thereby overcoming this problem as well.

Wilbur describes biotin containing compounds and biotinylation reagents where water soluble linker moieties that confer resistance to cleavage by biotinidase are incorporated. Wilbur also includes the synthesis and examples of application of water soluble multimers of biotin and means of amplifying selected targeting response by the multimers of biotin and biotin-binding proteins. Throughout the Wilbur document, the term "functional" refers to the organic chemical definition of reactive group or groups of atoms that render the structure of a particulate type of chemical or physical feature. The feature of Wilbur is the synthesis of various ionized and non-ionized linking moieties useful in preparing water soluble biotin derivatives. On page 11, a general scheme of non-ionized ether linked moieties useful for the preparation of water soluble biotin is described. While the biotin derivatives of Wilbur contain similar structures which play a part in the reagent of Applicants claimed invention, Wilbur does no teach or fairly suggest any core guidelines of Applicants' invention. Wilbur fails to teach or fairly suggest the presentation of a reagent capable of simultaneous binding of a defined ratio of affinity ligands and effectors to a biomolecule. Furthermore, Yau, Theodore, and Maddock all fail to remedy these deficiencies. Yau, Theodore, and Maddock all fail to teach or fairly suggest a reagent having three different

not commensurate scope of claims

moieties each serving a specific need of simultaneously labeling of targeting molecules for *in vivo* diagnostic and therapeutic applications in conjunctions with extracoporeal removal of non-targeted molecules from blood circulation. Yau, Theodore, and Maddock also fail to teach or fairly suggest a reagent having protection against biotinidase.

Yau and Theodore describe one type of pretargeting modality where avidin is administered to increase the rate of blood clearance prior to administration of small organic molecules comprising biotin and chelateradionuclide moieties. Yao and Theodore are only related to the present invention insofar that a biotin binding molecule is administered to form a complex with a biotinylated molecule. However, the reagent of Yao or Theodore is not labeled with and effector and there is no apparent need for a reagent of the type described in the present invention.

Maddock teaches a method of blood clearance by extracoporeal means. The use of an avidin/biotin system for such purposes is described as one of many different routes. Maddock does not give any details on how antibodies should be biotinylated nor does he give any guidance on the relation of effectors and affinity labels. Furthermore, there is nothing in Wilbur or Yau, Theodore, and Maddock which would motivate one of ordinary skill in the art to combine them as suggested by the Office Action.

The amendments to the claims and above remarks overcome the rejection. Thus, reconsideration and withdrawal are respectfully requested.

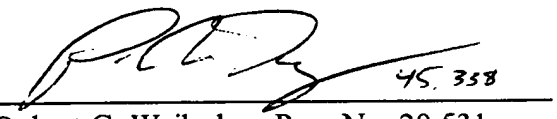
* * * * *

Applicants respectfully submit that this Amendment and the above Remarks overcome the outstanding objections and rejections in this case and place the application in condition for immediate allowance. Allowance of this application is earnestly solicited.

If any additional fees under 37 C.F.R. §§1.16 or 1.17 are due in this filing, please charge the fees to Deposit Account No. 02-4300; Order No. 033700.003.

Respectfully submitted,

SMITH, GAMBRELL & RUSSELL, LLP

By : 
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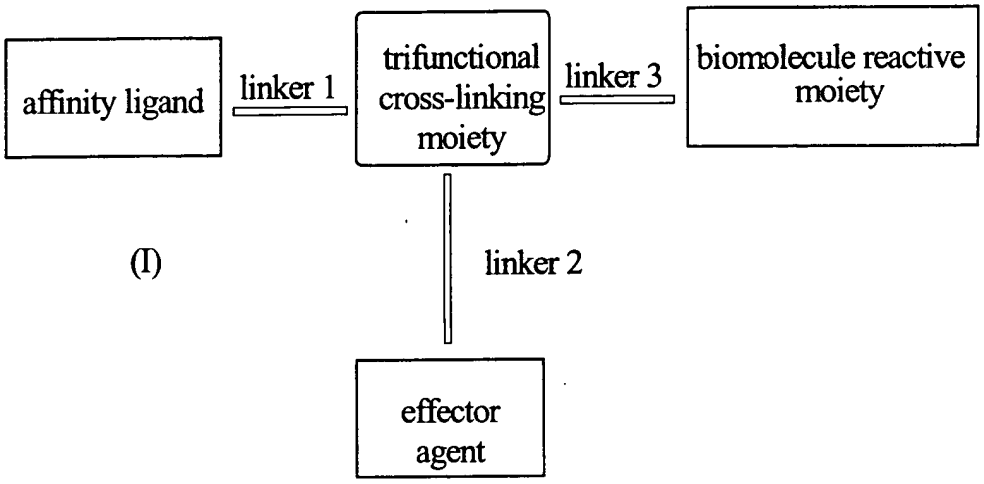
Dated : January 18, 2002

MARKED-UP VERSION OF THE AMENDED CLAIMS

1. (Amended) Reagent for conjugation to a biomolecule for diagnosis and treatment of human and animal conditions or diseases, wherein the reagent is a single molecule with at least three functional parts and has the following schematic structure (I):



(I)

a) wherein a trifunctional cross-linking moiety is coupled to

b) an affinity ligand via a linker 1, said affinity ligand being capable of binding with another molecule having affinity for said ligand which is stabilized towards cleavage by biotinidase of the biotinamide bond to release biotin to

c) an effector agent, optionally via a linker 2, said effector agent exerting its effect on cells, tissues and/or humorous molecules in vivo or ex vivo, and to

d) a biomolecule reactive moiety, optionally via a linker 3, said moiety being capable of forming a bond between the reagent and the biomolecule.

2. (Amended) Reagent according to claim 1, wherein the trifunctional cross-linking moiety is [chosen] selected from the group consisting of triaminobenzene, tricarboxybenzene, dicarboxyaniline and diaminobenzoic acid.

6. (Twice Amended) Reagent according to claim 1, wherein the biotin derivative is [chosen] selected from the group consisting of norbiotin, homobiotin, oxybiotin, iminobiotin, desthiobiotin, diaminobiotin, biotin sulfoxide, and biotin sulfone, or other molecules thereof that having essentially the same binding function.

7. (Amended) Reagent according to claim 5, wherein the stability towards enzymatic cleavage [preferably by bioinidase,] of the biotinamide bond to release biotin has been improved by using [biotin derivatives preferably] norbiotin or homobiotin.

8. (Twice Amended) Reagent according to claim 1, wherein linker 1 serves as an attaching moiety and a spacer between the trifunctional cross-linking moiety and the biotin moiety such that binding with avidin or streptavidin, or any other [biotin binding species] derivatives, mutants or fragments of avidin or streptavidin having essentially the same binding function to the affinity ligand, is not [diminished by steric hindrance] sterically hindered.

9. (Twice Amended) Reagent according to claim 1, wherein linker 1 contains hydrogen bonding atoms [such as ethers or thioethers], or ionizable groups [such as carboxylates, sulfonates, or ammonium groups] to aid in water solubilization of the biotin moiety.

10. (Amended) Reagent according to claim 1, wherein stability towards enzymatic cleavage[, preferably by biotinidase,] of the biotinamide bond to release biotin [have] has been improved by introducing an alpha carboxylate or an N-methyl group in linker 1.

11. (Amended) Reagent according to claim 1, wherein the effector agent is [chosen] selected from the group consisting of synthetic toxins, [or] natural occurring toxins, enzymes capable of converting a pro-drug to an active drug, immunosuppressive agents, immunostimulating agents, and radionuclide binding/bonding moieties, with or without the radionuclide.

14. (Twice Amended) Reagent according to claim 1, wherein the effector agent comprises aryl halides and vinyl halides for radionuclides of halogens, amino-carboxy derivatives[, preferably EDTA and DTPA derivatives, including Me-DTPA, CITC-DTPA, and cyclohexyl-DTPA], and cyclic amines[, preferably NOTA,. DOTA, and TETA] for In, Y, Pb, Bi, Cu, Sm, and Lu radionuclides.

15. (Twice Amended) Reagent according to claim 1, wherein the effector agent is provided with positron imaging radionuclides, [preferably F-18, Br-75, Br-76, and I-124;] therapeutic radionuclides, [preferably Y-90, I-131, In-114m, Re-186, Re-188, Cu-67, Sm-157, Lu-177, Bi-212, Bi-213, At-211, Ra-223;] and gamma imaging radionuclides[, preferably Tc-99m, In-111 and I-123].

17. (Twice Amended) Reagent according to claim 1, wherein linker 2 provides a spacer length of 1-25 atoms[, preferably a length of 6-18 atoms,] or groups of atoms.

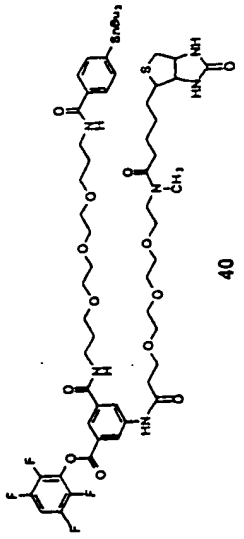
18. (Twice Amended) Reagent according to claim 1, wherein linker 2 contains hydrogen bonding atoms[, preferably ethers or thioethers], or ionizable groups[, preferably carboxylates, sulfonates, or ammonium groups,] to aid in water solubilization.

19. (Twice Amended) Reagent according to claim 1, wherein the biomolecule reactive moiety is [chosen] selected from the group consisting of active esters[, preferably N-hydroxy-succinimide esters, sulfo-N-hydroxysuccinimide esters, phenolic esters], aryl [and] or alkyl [imitates] imidates, alkyl or aryl isocyanates or isothiocyanates [reacting] reactive with amino groups on the biomolecule, [or] maleimides or alpha-haloamides [reacting] reactive with sulfhydryl groups on the biomolecule, [or] and aryl or alkylhydrazines or alkyl or aryl hydroxylamines [reacting] reactive with aldehyde or ketone groups naturally occurring or synthetically produced on the biomolecule.

21. (Twice Amended) Reagent according to claim 1, wherein linker 3 provides a spacer of a length of 1-25 atoms[, preferably a length of 6-18 atoms,] or groups of atoms.

22. (Twice Amended) Reagent according to claim 1, wherein linker 3 contains hydrogen bonding atoms [such as ethers or thioethers], or ionizable groups [preferably as carboxylates, sulfonates, or ammonium groups] to aid in water solubilization.

23. (Twice Amended) Reagent according to claim 1 wherein it is [chosen] <u>selected</u> from the group consisting of the following compounds:



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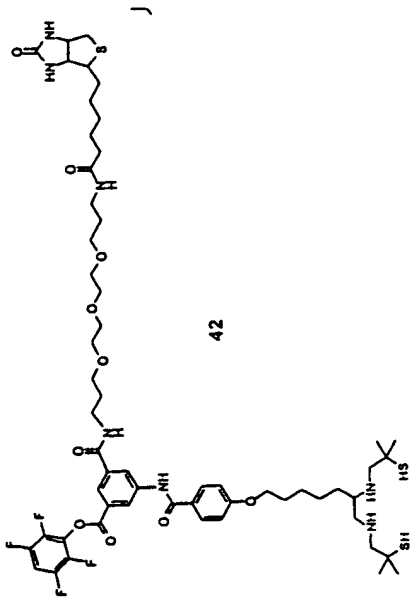

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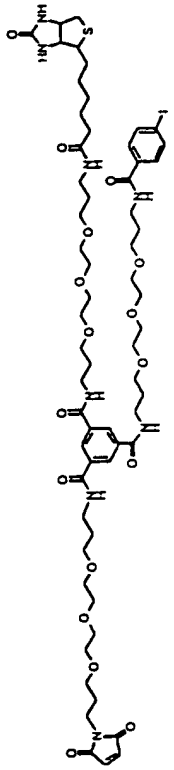

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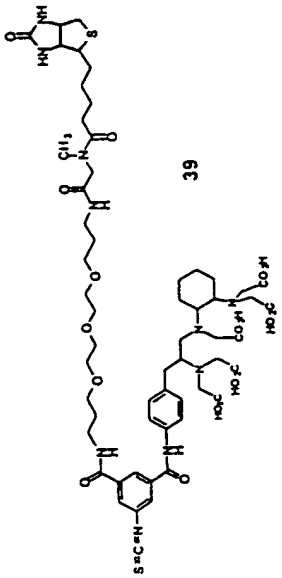

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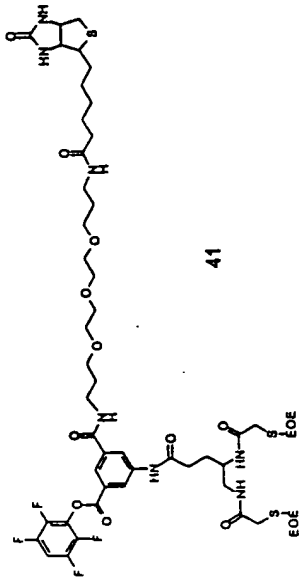

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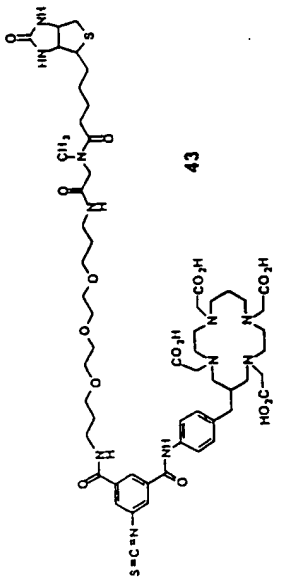

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, and

26. (Amended) Method for diagnosis or treatment of a mammalian condition or disease, wherein a reagent according to [any of the previous claims] claim 1 is conjugated to a biomolecule, and wherein said conjugated is added to the blood circulation of a mammal and kept therein for a certain of time in order to be concentrated to the target tissue or cells, wherein the biomolecules not being attached to the target tissue are completely or partially removed from the blood circulation by administration of a protein specifically binding to the affinity ligand or by passing the mammalian blood or plasma through an affinity column specifically adsorbing the conjugated biomolecule by specific interaction with the affinity ligand.

27. (Amended) Method for diagnosis or treatment of a mammalian condition or disease, wherein a reagent according to [any of the previous claims] claim 1 provided with a radionuclide is conjugated to a biomolecule, or alternatively, the reagent is conjugated to the biomolecule prior to attachment of the radionuclide, and the said radioactive conjugated biomolecule is added to the blood circulation of a mammal and kept therein for a certain period of time in order to be concentrated to the target tissue or cells, wherein the biomolecules that are not being attached to the target tissue are completely or partially removed from the blood circulation by administration of a protein specifically binding to the affinity ligand or by passing the mammalian blood or plasma through an affinity column specifically adsorbing the conjugated biomolecule by specific interaction with the affinity ligand.

28. (Amended) Kit for extracorporeally eliminating or at least reducing the concentration of a non-tissue-bound therapeutic or diagnostic biomolecule conjugate, which has been introduced to a mammalian host and kept therein for a certain time in order to be concentrated to the specific tissues or cells by being attached thereto, in the plasma or whole blood of the vertebrate host, said kit comprising a therapeutic or diagnostic biomolecule, a reagent for simultaneous conjugation of an affinity ligand and an effector agent to a biomolecule, means for extracoporeal circulation of whole blood or plasma from the vertebrate host, an optional plasma separation device for separation of plasma from blood, an extracoporeal adsorption device, and a means for return of whole blood or plasma without or with [low concentration] the remainder of non-tissue-bound target specific therapeutic or

diagnostic agent to the mammalian host, wherein the adsorption device comprises immobilized receptors specific towards an affinity ligand.

29. (Amended) A kit according to claim 28, wherein the effector agent is [chosen] selected from the group consisting of synthetic toxins, [or] naturally occurring toxins, enzymes, capable of converting a pro-drug to an active drug, immunosuppressive agents, immunostimulating agents, and radionuclide binding/bonding moieties with or without the radionuclide.